THE INTERACTION OF ORGANIC PHOSPHORUS INSECTICIDES

by

ERLINDA ROSARIO QUIAOIT

B. S., UNIVERSITY OF THE PHILIPPINES,
DILIMAN, QUEZON CITY, PHILIPPINES, 1953

A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Entomology

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1958

TABLE OF CONTENTS

INTRODUCTION

Research and development of organic insecticides have increased rapidly in an effort to find new and effective compounds against man's worst enemy, the insect. Many attempts have been made to produce the "one material" which may in effect control most of the known species of insect pests. However, after all the time and effort spent in the search for such a multipurpose insecticide, it is being realized that specific pests require specific compounds and formulations. Another problem man has to cope with is the diversity of habits of insects and their great capacity to adjust to anything new in their environment. These, added to their great reproductive potential, have enabled them to challenge many of the control measures used against them including the use of chemicals. Entomological literature has many instances of insects developing resistance to such potent insecticides as DDT, HCN, BHC, and other compounds.

One phase of the search for more potent weapons against the insect has been directed at the development of compounds which would enhance or extend the toxicity of insecticides already available. Joint action studies have yielded many encouraging results. However, most studies dealt with pyrethrum and its related compounds and not much work has been done with a more recent group of insecticides, the phosphorus compounds.

From the standpoint of insect control, combined activity of two or more pesticides is desirable. On the other hand, it may give rise to problems which do not end with the destruction of the insect. These are the problems associated with the use of the pesticides---to the warm-blooded animals that come in contact with them; to the workers who handle the insecticides; and to the consumer who would ingest a product which was treated with one or more insecticides.

Studies of phosphorus insecticides with the better known synergists have been carried out in this laboratory. Rai et al. (1956), Craig (1956), and Ware (1957) found instances of synergism and antagonism in their investigations using piperonyl butoxide in combination with malathion and other phosphorus compounds.

Recently, Frawley et al. (1957) have shown greater-than-additive toxic effects of two phosphate pesticides when administered simultaneously to rats or dogs.

This investigation was undertaken to determine the effects of such combinations when applied topically using the house-fly as test insect.

LITERATURE REVIEW

Extensive studies have been done regarding the use of supplementary, auxiliary, or synergistic materials in insecticidal formulations and many theories in connection with their mode of action formulated. The terms "activation" and

"synergism" have been employed widely to signify increased activity of mixtures of insecticides.

In 1929, Inman applied the term "activator" to an increase in the physical efficiency of nicotine by the addition of a compound which would release the free volatile nicotine base from nicotine sulfate sprays. Macht (1929) defined synergism as the

> phenomenon exhibited by the combination of two or more drugs in which the pharmacodynamic effect produced by the mixture is not a simple summation of the effects produced by two or more individual components. Such combinations produce a pharmacological effect of an unexplained nature in that the effect of one component may be greatly heightened or potentiated by the other or in other cases an antagonistic action of one drug over the other may be shown.

This definition covers both potentiation and synergism.

Synergism is usually defined as a joint action of two materials such that the total effect is greater than the sum of the two effects when used alone (Wadley, 1945, 1949). Horsfall (1945) took a different view of synergism and limited it to joint actions which are not antagonistic and thus included additive action. He also divided it into supplementary and potentiated types, the former included such effects as physical improvement of the properties of the poison.

Bliss (1939) distinguished between simple additive and synergistic action and defined three possible physiological types of joint action, namely, synergistic, independent, and similar. He considered antagonism as negative synergism. Finney (1947), Hewlett and Plackett (1952) concurred with his concepts.

Sumerford (1954) used synergism and potentiation interchangeably to describe the condition wherein the "combination had a higher toxicity than either component used alone." Lately, "potentiation" has been used in a limited sense as the "greater-than-additive toxic effect of two phosphate pesticides administered simultaneously" (Cook et al., 1957; Frawley et al., 1957).

Most synergism studies have dealt with pyrethrum and its related compounds. Schmidt (1955) has an excellent review on the subject. Lindquist et al. (1947), Brown (1951), and Metcalf (1948, 1955) also reviewed the data regarding pyrethrin synergists. Roark (1952, 1955), Eddy et al. (1954) discussed the synergists of allethrin, a synthetic homolog of pyrethrin.

March et al. (1952) undertook a search for synergists for chlorinated hydrocarbons against DDT-resistant houseflies. Sumerford et al. (1951, 1954) ran tests for a potent synergist for DDT and examined 200 halogen-containing synergists and insecticides. Pal (1951) observed synergism between DDT and BHC in combined sprays against houseflies and mosquitoes.

When organic phosphates came to use no investigations were made to study the effect of synergists until Eddy et al. (1954)

reported the results of their studies of synergists which included three organic phosphates against the body louse, Pediculus humanus corporis (Deg.). In those cases, the initial kill and residual effectiveness were greatly increased. Hoffman et al. (1954) tested the effectiveness of several organic phosphorus compounds in combination with synergists against DDT-resistant houseflies by residue studies and found all the synergists effective except with malathion.

Rai et al. (1956) found that piperonyl butoxide and malathion were highly antagonistic to both susceptible and DDT-resistant strains of houseflies. Craig (1956) noted the antagonistic action of the combination against the German roach, Blatella germanica (L.). A subsequent study was made by Ware (1957) to determine the effect of piperonyl butoxide with the different analogs of malathion. He found that the oxygen analogue was synergized by piperonyl butoxide.

Only a few studies have been made regarding the effect of combinations of two or more organic phosphorus insecticides. Frawley and Fuyat (1957) investigated the effect of sub-acute level feedings of parathion and Systox in dogs by measurements of plasma-cholinesterase changes. When both insecticides were in the same diet, the effect on cholinesterase was at least additive.

Cook et al. (1957) worked on the enzymatic hydrolysis of malathion and its inhibition by EPN and other organic phosphorus compounds in an effort to explain the biochemical basis for potentiation. L. F. Lewis (1957)[1] summarized the results

1. Personal Communication

using houseflies as test insects for potentiation studies of several phosphorus compounds. In tests with the materials as dry sugar baits and by topical application, a majority of combinations caused greater mortality than expected from either compound alone and compounds ET-57, Dipterex, malathion, Diazinon, and EPN appeared to be somewhat more susceptible to potentiation than other materials used.

MATERIALS AND METHODS

The test insect used in these experiments was the DDT-susceptible (KUN 48) strain of housefly, Musca domestica (L.) which was secured from the University of Kansas, Lawrence, Kansas. The flies were reared under a modified CSMA system and were not exposed to any insecticide during the rearing process. Adult flies were fed on a 2:1 mixture of powdered milk and granulated sugar. Water-saturated cellucotton in "Dixie" cups was provided for water supply as well as for egg-collection since the flies readily oviposited on this wet material.

Insect Manipulation, Sexing, and Counting

Only four-day-old male flies were used in the tests. These were separated from the females three days after emergence from the pupal stage and preconditioned for 24 hours at a temperature of 80°±2°F. before treatment.

The flies were taken out of the rearing cages by means of suction. Cylindrical cardboard cartons of quart size, the ends

of which were replaced with a piece of wire screen mesh, were used as containers. One end was fitted into the suction end of a blower and the other end where the lid fits was appressed to the cage opening. A light bulb placed behind the carton attracted the flies and tapping the sides of the rearing cages facilitated the transfer.

The flies were brought to the laboratory for sexing. They were lightly anaesthetized with carbon dioxide. The males were picked up with a pair of forceps and placed into the recovery jars. About 100 to 110 male flies were placed in each jar. The females were either returned to the cages or drowned in technical acetone.

Food, consisting of evaporated milk diluted with an equal amount of water, was provided. A piece of cellucotton was dippted into the milk solution and held in a 3/4 ounce souffle cup placed in each jar.

The jars were covered with a piece of cheesecloth held in place by a rubber band. The cheesecloth was disposed of after each use.

Weighing

The average weight of the flies was taken in every test. Fifty preconditioned flies were taken from jars, mildly anaesthetized for counting and placed into a glass tare. An analytical balance was used for weighing. The flies used in weighing were also used in the treatments.

Chemicals and Solvents

All insecticides used were organic phosphates. EPN was used in combination with malathion, Thimet, and American Cyanamid compounds 4124 and 4389.

EPN, ethyl-p-nitrophenyl benzene thiophosphonate, 98 per cent pure crystalline solid was supplied by DuPont de Nemours and Co., Inc., and was stored in a desiccator.

Malathion, 0, 0-dimethyl S-(1,2-dicarboethoxy-ethyl) dithiophosphate, 99.6 per cent; Thimet, 0, 0-diethyl S-(ethylthiomethyl) phosphorodithioate; experimental insecticides 4124, 0,0-dimethyl 0-(2 chloro-4 nitrophenyl) thiophosphate, and 4389, the oxygen analogue of malathion were obtained from the American Cyanamid Company.

The solvent used for all the dilutions was glass-distilled technical grade acetone. This was also used for rinsing all glassware and equipment needed for the experiments.

Apparatus

A microapplicator designed by Roan and Maeda (1953), was used in all topical applications. The syringe was calibrated with mercury to eject 1.08 microliters of toxicant for each movement of the micrometer. Only one syringe was used in all the tests.

Flies were kept before and after treatments in a constant temperature chamber. The chamber used consisted of a double door, 20-cubic-foot refrigerator converted by the addition of

a controlled heating unit. All tests were conducted at $80^{o} \pm 2^{o}$F. and room humidity.

Preparation of Solutions

The solutions of toxicant were prepared about an hour before treatments were made. Empty glass vials were first weighed on an analytical balance. Then the required weight of insecticide was added to the weight of the vial and the vial filled with insecticide. A spatula was used for transferring dry samples and a finely drawn capillary dropper for the liquid ones. Ten mg. was sufficient for each sample. Desired dosages were made from these weighed samples by dilution with the appropriate amount of solvent.

Determination of the LD_{50}

For the determination of the LD_{50} of each compound, it was necessary to establish a range of concentrations which would give from 10 to 90 per cent mortality. Four to five different dosages were used and three to eight replicates were run. The values obtained were plotted on probit-mortality graphs. The LD_{50} for each toxicant was based on the corrected amount of insecticide applied and the average weight of the flies.

Potentiation Studies

Solutions to be used later in combination were prepared to contain the LC_{50} and the LC_{10} values of the toxicant.

The first test required a mixture of 1/2 LC_{50} of each of the compounds. The second solution was a mixture of 1/2 LC_{10} of EPN and 1/2 LC_{10} of the other toxicant. If the results of the above were more than additive, additional tests were carried out employing the following design:

1/5 LC_{10} EPN + 1/5 LC_{50} of other compound
1/10 " " 1/10 " " "
1/20 " " 1/20 " " "

To determine which compound was responsible for potentiation, the following design was used:

1/10 LC_{50} EPN + 1/20 LC_{50} of other compound
1/20 " " 1/10 " " "

All the toxicants were mixed before the start of treatment.

Insecticide Application

Prior to treatment, recovery jars were prepared to hold the treated flies by placing in each pint jar a souffle cup containing a wad of cellucotton saturated with diluted evaporated milk. Pieces of cheesecloth were used to cover the jars.

All applications of insecticides were done topically on the mesosternum of the fly. The syringe was filled to its 0.25 cc. capacity with the toxicant solution or mixture, any air bubbles were expelled, and the syringe then mounted on the microapplicator. A few movements of the ratchet were made to clear the apparatus and to check for a constant flow of the solution.

The flies were taken out of the constant temperature chamber a jar at a time. Dead and weak flies were separated and the live ones anaesthetized mildly with carbon dioxide.

A pair of forceps was used in handling the flies. The blunted tip of the needle of the syringe was brought into contact with the mesosternum and the ratchet pulled down to deliver the required amount of toxicant. Each fly was allowed to stay in contact with the needle tip for a few seconds to allow for proper dispersal of toxicant on the thorax. As a precautionary measure, the insect was always held with its head up to avoid spilling of the toxicant into the mouthparts. Also, care was taken not to drop the unconscious fly into the wet cellucotton since flies with wet wings are sometimes trapped on the glass walls and die of exhaustion.

Fifty flies were treated with each concentration and 25 flies were kept in each jar. The jars were returned immediately after labelling to the constant temperature box and kept there another 24 hours for mortality observations.

Lower concentrations were applied first, the strength of the toxicant increasing with each succeeding dosage. The syringe and needle were thoroughly washed with distilled acetone, air dried with a hand blower, then rinsed two or three times with the next solution before filling the syringe again.

Mortality Readings and Control

The dead flies were counted 24 hours after treatment. A dead fly was one which showed no movement of legs or proboscis when touched or subjected to other stimulation.

Two jars containing 25 untreated flies each were set aside for control. Earlier in the experiment, the flies were treated with glass-distilled acetone, but this was discontinued when it was found that they were not affected by it. Previous workers in this laboratory also had the same results. Whenever dead flies were found in the control jars, true mortality was corrected using Abbot's formula (1925).

RESULTS AND DISCUSSION

A summary of the data obtained can be found in Table 1. The LC_{50} and LC_{10} values of each compound used, the various combinations levels tested and the corresponding mortality values are also given.

The dosage-mortality curve for each toxicant used is given in Plate I. These values represent the average of four to eight replicates that were made for each compound. The concentrations are expressed in micrograms per microliter, and the actual amounts applied per insect was 1.08 times these values as the syringe used was calibrated to eject this amount on each application.

The data obtained were analyzed according to Wadley's procedure (1945, 1949) for determining the presence of synergism

between two compounds. Since EPN was used in all the mixtures, the concentrations of the other compounds were calculated in terms of their EPN equivalence. A predicted effect can then be interpolated from the EPN dosage-mortality curve which can be compared with the actual values obtained.

A comparison between the actual and expected mortality values are shown on Tables 2, 3, 4, and 5 and Plates II and III. It seems evident that there is a certain degree of interaction between the insecticides used. In all the binary mixtures studied, there was a significant shift of the line to the left of the predicted mortality line indicating the presence of synergism of greater-than-additive effect of the mixture. When one-half the amounts of the toxicant needed to kill 50 per cent of the population tested were mixed, the percentage kill in each case was better than 50 per cent, which shows a greater-than-additive effect. This is also the result obtained when half of the amounts of the lethal concentration to kill 10 per cent is used.

The last two columns of Table 1 give the summary of the result of the tests to determine which compound was responsible for potentiating the other. It appears that both compounds affect each other in some way. The first combinations, EPN with malathion and EPN with compound 4389, gave higher mortalities when EPN was at a lower concentration. The reverse was true, however, with the EPN-Thimet and EPN-compound 4124 mixtures. This seems to indicate that in the first two cases, EPN was responsible for the increased effect but not in the

last two cases.

It has been known that mixtures of insecticides may act similarly, independently or synergistically (Bliss, 1939). In cases of independent action, the components are assumed to act at different reactor sites producing unrelated effects and the insect dies from the effect of one or the other and not as a result of the cumulative or joint effect of the poison. If the action is similar, the toxicants act upon the same physiological system when applied separately and produce the same response when applied jointly. However, when the poisons are simultaneously applied and the result produced is greater than what is expected, a synergistic effect is indicated.

Storrs and Burchfield (1954) in an investigation of a series of 14 binary mixtures found malathion and parathion to produce similar action when applied to mosquito larvae.

Frawley et al. (1957) found up to 50-fold potentiation in the acute toxicity of EPN and malathion if administered simultaneously to dogs, but the potentiation was less for rats. They also suggested that the potentiation may be different if a different ratio between the two compounds were employed. In a later work, Cook et al. (1957) tried to elucidate on the mechanism of potentiation between these two compounds and found that EPN blocked the destruction of malathion in liver homogenates.

The compounds used in this study have a common physiological action in that they are all known to be cholinesterase inhibitors in various animals. It can be expected, therefore,

to have at least a similar joint action when binary mixtures of these compounds are applied to the test insect. However, a greater-than-additive effect was actually obtained, which suggested the presence of an interaction between the component chemicals in the mixture.

Table 1. The LC_{50} and LC_{10} values of the organic phosphorus insecticides[1] used and the average per cent mortalities of combinations at different levels on a susceptible strain of houseflies[2].

Toxicant in combination with EPN	LC_{50} ug/ul	LC_{10} ug/ul	Per cent Mortality[3]: 1/2 LC_{50} + 1/2 LC_{50}	1/2 LC_{50} + 1/2 LC_{10}	1/5 LC_{50} + 1/5 LC_{50}	1/10 LC_{50} + 1/10 LC_{50}	1/20 LC_{50} + 1/20 LC_{50}	1/10 LC_{50} EPN + 1/20 LC_{50}	1/20 LC_{50} EPN + 1/10 LC_{50}
EPN	0.03	0.0215							
Malathion	0.24	0.15	78.4	25.3	21.7	17.0	8.6	7.0	11.6
Cpd. 4389	0.194	0.10	68.8	23.2	14.0	9.4	5.3	12.4	14.0
Thimet	0.082	0.05	64.0	22.4	15.4	9.4	6.6	12.0	7.0
Cpd. 4124	0.021	0.014	65.0	21.0	9.0	6.4	3.5	10.0	5.0

1/ Actual amount applied 1.08 times dosages indicated.

2/ Average weight 15.0 ± 0.8 mg/fly. Four-day-old male flies only.

3/ Corrected for control mortality by Abbot's (1925) formula.

EXPLANATION OF PLATE I

Dosage-mortality curves for KUN 48 house flies topically treated with various organic phosphorus insecticides. Concentration of toxicants in micrograms per microliter.

Legend:		
	A---A	American Cyanamid Compound 4124
	B---B	EPN
	C---C	Thimet
	D---D	American Cyanamid Compound 4389
	E---E	Malathion

PLATE I

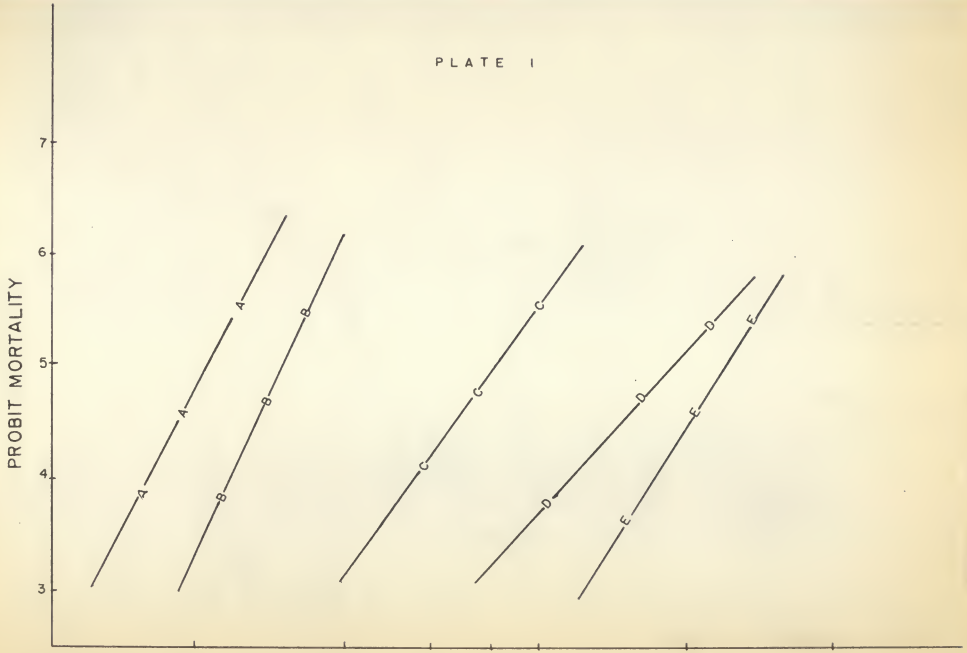

PROBIT MORTALITY
7
6
5
4
3
A
A
A
A
B
B
B
B
C
C
C
C
D
D
D
D
E
E
E
E
.02
.04
.06
.08
.1
.2
.4
LOG DOSAGE

Table 2. The actual and interpolated mortalities obtained with a mixture of EPN and malathion at various concentrations. 1/

EPN Conc. ug/ul	Malathion Conc. ug/ul	EPN Equivalence 2/ ug/ul	Mortality (%) Actual	Mortality (%) Interpolated 4/
0.015	0.12	0.03	78.4	50.1
0.011	0.08	0.019	22.4	6.06
0.006	0.05	0.012	17.2	< 1

Table 3. Actual and interpolated mortalities of a mixture of EPN and 4389 at various levels. 1/

EPN Conc. ug/ul	4389 Conc. ug/ul	EPN Equivalence 3/ ug/ul	Mortality (1%) Actual	Mortality (1%) Interpolated 4/
0.015	0.097	0.029	68.8	50.0
0.011	0.050	0.018	23.2	5.01
0.006	0.039	0.012	14.0	< 1

1/ Actual amount applied is 1.08 times dosage indicated.

2/ EPN + 0.365 (malathion).

3/ EPN and 0.145 (compound 4389)

4/ See Figure 1, EPN slope.

EXPLANATION OF PLATE II

Dosage-mortality curves on KUN 48 house flies for binary mixtures of organic phosphorus insecticides showing expected and actual mortality values. Toxicant dosages in micrograms per microliter are expressed as EPN-equivalents.

Fig. 1. EPN + Malathion

Fig. 2. EPN + compound 4389

Legend: X---X Actual mortality

O---O Expected mortality

PLATE II

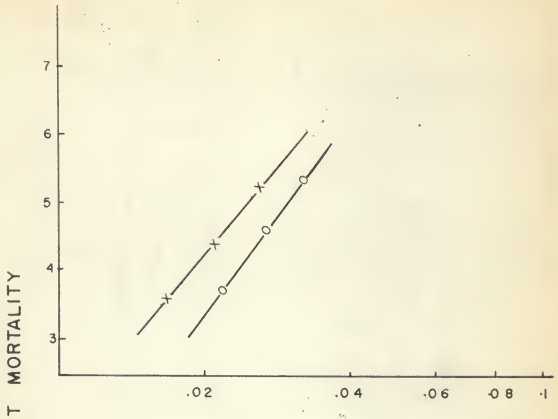


FIG. 1

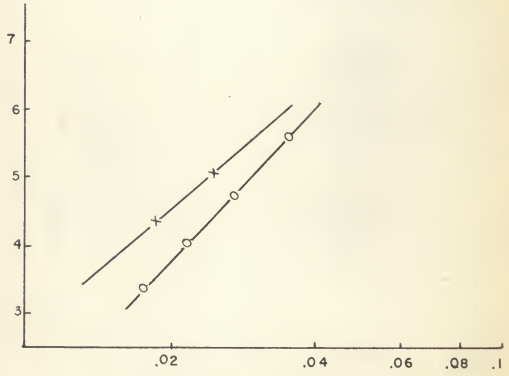


FIG. 2

Table 4. The actual and interpolated mortalities from a mixture of EPN and Thimet at various concentrations. 1/

EPN Conc. ug/ul	Thimet Conc. ug/ul	EPN Equivalence 2/ ug/ul	Mortality (1%) Actual	Mortality (1%) Interpolated 4/
0.015	0.041	0.030	64	50
0.011	0.025	0.02	22.4	6.06
0.006	0.0164	0.012	15.4	1

Table 5. Actual and interpolated mortality values for a mixture of EPN and compound 4124 at various levels. 1/

EPN Conc. ug/ul	Compound 4124 Conc. ug/ul	EPN Equivalence 3/ ug/ul	Mortality (1%) Actual	Mortality (1%) Interpolated 4/
0.015	0.011	0.030	65	50
0.011	0.007	0.021	21	10
0.006	0.004	0.012	9	1

1/ Actual amount applied 1.08 times more than dosage indicated.

2/ EPN + 0.365 (Thimet).

3/ EPN and 1.41 (compound 4124).

4/ See Fig. 1, EPN slope.

EXPLANATION OF PLATE III

Dosage-mortality curves on KUN 48 house flies for binary mixtures of organic phosphorus insecticides showing expected and actual mortality values. Toxicant dosages in micrograms per microliter are expressed as EPN-equivalents.

Fig. 1. EPN + Thimet

Fig. 2. EPN + compound 4124

Legend: X---X Actual mortality

O---O Expected mortality

PLATE III

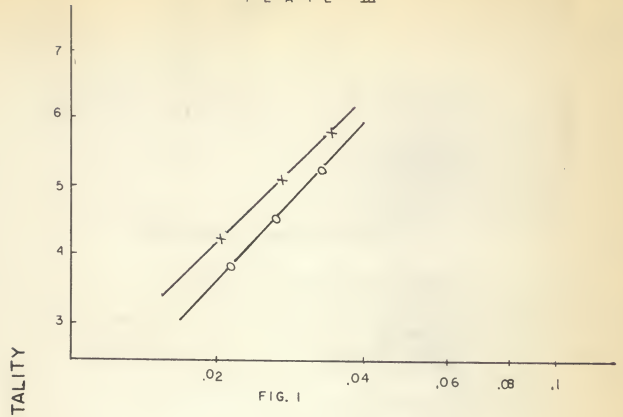
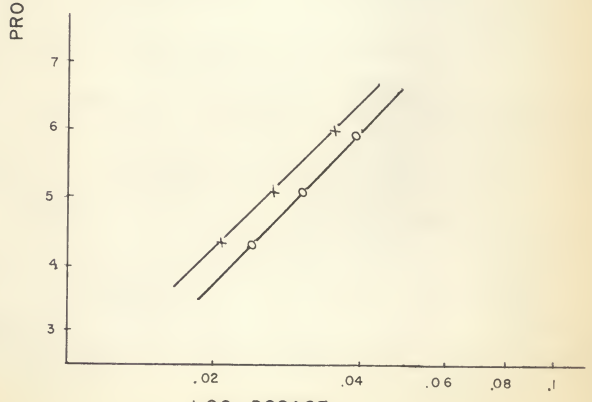


FIG. 1

PROBIT MORTALITY



LOG DOSAGE

FIG. 2

CONCLUSIONS AND SUMMARY

The effect of binary mixtures of organic phosphorus insecticides were studied by using four-day-old male houseflies of a susceptible strain. Combinations of EPN and malathion, American Cyanamid Compounds 4124 and 4389, and Thimet at various fractions of their LC_{50}'s were topically applied on the mesosternum of the housefly by a microdevise.

The data obtained seem to indicate the presence of an interaction between the toxicants. Although the data were not statistically analyzed, there is suggested a certain degree of potentiation in all the combinations studied. However, the extent to which the compounds are potentiated vary at different levels, which makes a comparison difficult. There is an indication that EPN was responsible for the increased activity of malathion and the malathion analogue, compound 4389, but its activity was supplemented by Thimet and compound 4124.

There has been so little work done along this line of investigation that conclusive evidence of potentiation or synergism among and/or between compounds used in this experiment could not be derived. This investigation is a preliminary work and much more should be done to answer the question: "Is there potentiation of binary mixtures of organic phosphorus insecticides?" This preliminary work, in spite of the fact that no conclusive answer could be derived, opens avenues

toward a better and different approach to the problem. A more thorough investigation should be undertaken regarding the possible modes of action.

ACKNOWLEDGMENTS

The writer wishes to express her gratitude to Drs. Clifford C. Roan and Lallan Rai, advisers, for their guidance and valuable criticisms during the course of the experiment and preparation of the manuscript.

To Dr. Herbert Knutson, head of the Department of Entomology, gratitude is expressed for editing the manuscript and for helping make it possible for the writer to come to America and do graduate work by aiding in obtaining an assistantship; and to Dr. Fred Lawson for permitting the writer time to complete the M. S. thesis requirements while assigned on an assistantship on another project; and to the many others who helped in one way or another, many thanks to them.

REFERENCES

Abbot, W. S.
A method of computing the effectiveness of an insecticide. J. Econ. Ent. 18: 265-267. 1925.

Bliss, C. I.
The toxicity of poisons applied jointly. Annals Appl. Biol. 26: 585-616. 1939.

Bourne, A. I. and W. D. Whitcomb.
Investigation of materials which promise value in insect control. Bull. Mass. Agr. Expt. Sta. 327: 39-40. 1936.

Brown, A. W. A.
Insect control by chemicals. New York: John Wiley and Sons. 817 pp. 1951.

Bushland, R. C., G. W. Eddy and E. F. Knipling.
Tests with synergists for pyrethrin against the body louse. J. Econ. Ent. 37: 556-567. 1944.

Cook, J. W., J. R. Blake, and M. W. Williams.
The enzymatic hydrolysis of malathion and its inhibition by EPN and other organic phosphorus compounds. J. of Ass. of Official Ag. Chem. 664-665. 1957.

Craig, James.
A comparative study of the toxicity of malathion and malathion plus piperonyl butoxide by topical application on the German cockroach, B. germanica (L.) and Madera cockroach, L. maderae (F.). Master's thesis, Kansas State College, 1956.

Eddy, G. W., M. M. Cole, and A. S. Marulli.
Tests of synergists with phosphorus compounds against the body louse increased the initial activity ten times. Soap and Chem. Spec. 30: 121-123. 1954.

Finney, D. J.
Probit analysis. Univ. Press. Cambridge, England. 256 pp. 1947.

Frawley, J. P. and H. N. Fuyat.
Effect of low dietary levels of parathion and systox on blood cholinesterase of dogs. Ag. and Food Chem. 5 (5): 346-348. 1957.

Frawley, J. P., H. N. Fuyat, E. C. Hagan, J. R. Blake, and O. G. Fitzhugh.
Marked potentiation in Mammalian toxicity from simultaneous administration of two anticholinesterase compounds. In press.

Hewlett, P. S. and R. L. Plackett.
Similar joint action of insecticides. Nature 169 (4292): 199. 1952.

Hoffman, R. A., T. L. Hopkins, and A. W. Lindquist.
Tests with pyrethrin synergists combined with some organic phosphorus compounds against DDT-resistant flies. J. Econ. Ent. 47: 72-74. 1954.

Horsfall, J. J.
Fungicides and their action. Waltham, Mass. 1945.

Inman, M. T.
Sulfonated oxidation products of petroleum as insecticide activators. Ind. Eng. Chem. 21: 542-543. 1929.

Lindquist, A. W., H. H. Madden, and H. O. Wilson.
Pretreating houseflies with synergist before applying pyrethrin sprays. J. Econ. Ent. 40:426-427. 1947.

Macht, D. T.
Pharmacological synergism of stereisomers. Proc. Natl. Acad. Sci. U. S. 15: 63-70. 1929.

March, R. B., R. L. Metcalf, and L. L. Lewallen.
Synergists for DDT against insecticide-resistant houseflies. J. Econ. Ent. 45:851-860. 1952.

Metcalf, R. L.
The mode of action or organic insecticides. Natl. Res. Connc., Natl. Acad. Sci., Chem. Bio. Coord. Center Rev. No. 1. 84 pp. 1948.

_____.
Insects vs. insecticides. Sci. Amer. 187: 21-25. 1952.

_____.
Organic insecticides. New York: Interscience. 392 pp. 1955.

Pal, Rajinder.
Synergistic action of DDT and BHC combined sprays. Nature 167 (4244): 368. 1951.

Rai, L., S. E. D. Afifi, H. C. Fryer, and C. C. Roan.
The effects of different temperature and piperonyl butoxide on the action of malathion on susceptible DDT-resistant strains of houseflies. J. Econ. Ent. 49 (3): 307-310. 1956.

Roan, C. C. and S. Maeda.
A microdevise for rapid application of toxicants to individual insects. U. S. D. A. Bur. Ent. Plant Quar. ET 306. 3 pp. 1953.

Roark, R. C.
A digest of information on allethrin. U. S. D. A., Bur. Ent. Plant Quar. E-846. p. 58. 1952.

_____.
A second digest of information on allethrin and related compounds. U. S. D. A., Agr. Res. Ser. ARS-33-12. p. 33 1955.

Schmidt, C. H.
Studies of synergists for pyrethrins and related synthetic compounds. Ent. Soc. Amer. No. Cent. States Br. Proc. 10: 56-60. 1955.

Shepard, H. H.
The chemistry and action of insecticides. 1st. edition. McGraw-Hill Book Co., Inc. New York 1951.

Storrs, E. E. and H. P. Burchfield.
Joint action of binary mixtures of insecticides. Contrib. Boyce Thompson Inst. 18 (1): 69-78. 1954.

Sumerford, W. T.
Synergism and synergists. Review of synergism among halogen-containing synergists. Ag. and Food Chem. 2 (6): 310-327. 1954.

Sumerford, W. T., M. B. Goette, K. D. Quarterman, and S. L. Schenck.
The potentiation of DDT against resistant houseflies by several structurally related compounds. Science 114: 6-7. 1951.

Wadley, F. M.
The evidence required to show synergistic action of insecticides and a shortcut in analysis. U. S. D. A. Bur. Ent. and Plt. Quar. ET 223. p. 7. 1945.

Wadley, F. M.
Shortcut procedure for error estimate in laboratory studies of synergism in insecticides. U. S. D. A. Bur. Ent. and Plt. Quar. ET 275. p. 5. 1949.

Ware, George.
The interaction of malathion and several analogs with piperonyl butoxide applied topically to male houseflies. Ph. D. dissertation. Kansas State College. 1957.

THE INTERACTION OF ORGANIC PHOSPHORUS INSECTICIDES

by

ERLINDA ROSARIO QUIAOIT

B. S., UNIVERSITY OF THE PHILIPPINES,
DILIMAN, QUEZON CITY, PHILIPPINES, 1953

AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Entomology

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1958

In an effort to find more potent weapons against his enemy, the insect, man has concentrated much of his efforts to the development of insecticides. Every year witnesses the birth of a new compound upon which thousands of dollars would be poured for its development. An extensive exploitation of its potentialities is undertaken, hoping this may be the "one material," the answer, the weapon he badly needs in his hope to end forever the great biological battle between him and the insects. But sooner or later, the insect turns around, and with its great reproductive potential and capacity to adjust, challenges the same material by developing resistance to it.

One phase of the search has been directed at the development of compounds which would enhance or extend the toxicity of insecticides already available. Joint action studies have yielded many encouraging results. However, most studies have dealt with pyrethrin and its related compounds and so very little work has been done with a more recent group of insecticides, the phosphorus compounds.

Combined activity of two or more pesticides is desirable from the standpoint of insect control. However, this also gives rise to another problem which does not end with the destruction of the insects. These are the hazards which are associated with the use of such pesticides to warm-blooded animals that come in contact with them, to the workers who handle the insecticides, and to the consumer who will use the treated product.

Quite a number of workers have studied the effects of supplementary, auxiliary, synergistic, or potentiating materials in insecticidal formulations. Some have done research along this line using organic phosphorus compounds and the better known adjuncts like piperonyl butoxide. But only a few studies have been made regarding the effect of combinations of two or more of these insecticides themselves. These experiments were conducted in an effort to determine whether or not there would be any interaction between organic phosphorus insecticides when applied simultaneously to the test insect.

The test insect used in these experiments was the DDT-susceptible (KUN 48) strain of housefly, Musca domestica (L.). They were reared under a modified CSMA method in a constant temperature rearing room. Only four-day-old male flies were used in the tests. The flies were separated from the females three days after emergence from the pupal stage, placed in pint jars and preconditioned for 24 hours at a temperature of 80° plus or minus 2° F. and at room humidity before treatment. Carbon dioxide was used to anaesthetize them. Food in the form of evaporated milk diluted with an equal amount of water was provided during the preconditioning period and after treatment. The flies were weighed shortly after treatment.

The compounds used were all organic phosphorus compounds. EPN, ethyl-p-nitrophenyl benzene thiophosphonate, was used in combination with each of the following compounds: malathion, O-O-dimethyl S-(1-2-dicarboethoxyethyl) dithiophosphate;

Thimet, O-O-diethyl S-(ethylthiomethyl) phosphorodithioate; American Cyanamid compounds 4124, O-O dimethyl O-(2 chloro-4 nitrophenyl) thiophosphate; and 4389, the oxygen analogue of malathion. The solvent used for all dilutions was glass-distilled technical grade acetone.

The LD_{50} and LD_{10} values were first determined for each compound by establishing a range of concentrations which would give 10 to 90 per cent mortality. Binary mixtures tested contained the following concentrations: 1/2 LC_{50} EPN and 1/2 LC_{50} of other compound; and 1/2 LC_{10} EPN and 1/2 LC_{10} of other compound. If the above mixtures were greater-than-additive in effect, additional tests were carried out with solutions containing 1/5, 1/10, and 1/20 each of the LC_{50} values of the toxicant being studied.

To determine which of the toxicants was responsible for potentiation, the following design was employed: 1/10 LC_{50} EPN and 1/20 LC_{50} of other compound; and 1/20 LC_{50} and 1/10 LC_{50} of other compound.

All toxicants were diluted and/or mixed prior to treatment. Applications were done topically on the mesosternum of the fly by means of a calibrated syringe mounted on a microapplicator device. Lower concentrations were used first and care was taken to avoid contamination of the solutions. Fifty flies were treated with each solution for every replicate and four to eight replicates were run.

The flies were then returned to the constant temperature chamber. Mortality readings were taken at the end of 24 hours.

For control, two jars containing 25 untreated flies were set aside. Abbot's formula was used to correct mortality when dead flies were found in the control.

The data obtained were analyzed according to Wadley's (1945, 1949) procedure for determining the presence of synergism between two compounds. Since EPN was used in all the mixtures, the concentration of the compounds were calculated in terms of their EPN equivalence. A predicted effect can then be interpolated from the dosage-mortality curve of EPN which can be compared with the actual mortality values obtained. This was based on the assumption that all these compounds acted similarly, they being cholinesterase inhibitors and, therefore, have a common physiological effect.

A greater-than-additive effect was observed in all the cases studied when binary mixtures were used thus indicating the presence of an interaction between the components. There is an indication that EPN was responsible for the increased activity of malathion and its analogue, American Cyanamid compound 4389, but was supplemented by Thimet and American Cyanamid compound 4124.